Vernonia anthelmintica (L.) Willd.: (+) and (−)-*Threo*-12,13-dihydroxyoleic Acid

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The occurrence of 12,13-epoxyoleic acid in seed oils was first characterised by Gunstone[1] who identified it in *Vernonia anthelmintica* (L.) Willd. by converting the epoxy acid to the 12,13-dihydroxyoleic acid. Bharucha and Gunstone[2] pointed out that the epoxy acid has two asymmetric centres and therefore may be expected to occur in enantiomorphic forms. Chisholm and Hopkins[3] obtained two optically active *threo*-12,13-dihydroxyoleic acids, the (+)-isomer from the seed oil of *Malope trifida*, Cav.[3] and species of the Malvaceae family[4] and the (−)-isomer from the seed oil of *Vernonia colorata*, Drake, both by the acetolysis procedure used by Gunstone.[1,2] Miwa and co-workers[5] reported that (+)-*threo*-dihydroxyoleic acid appeared in the maturing *V. anthelmintica* seed with onset of accelerated growth as a precursor of 12,13-epoxyoleic acid but that in later stages it completely disappeared. The treatment of seed as described in our work appears to represent a reversal of the biosynthesis described by Miwa *et al.*[5]

In the present work these optically active *threo*-12,13-dihydroxyoleic acids have been obtained from *V. anthelmintica*. The (+)-*threo*-12,13-dihydroxyoleic acid was obtained initially from the mature seed by a hydrating principle (possibly enzymatic). The (−)-*threo*-12,13-dihydroxyoleic acid was prepared by acetolysis[1,2] followed by repeated crystallisations which Chisholm and Hopkins[3] found necessary in order to obtain optical purity, since the conversion of epoxy to glycol by acetolysis is only partially stereospecific. The present work is also the first in which the isolation of the pure (−)-isomer from *V. anthelmintica* is reported.

(+)-*Threo*-12,13-dihydroxyoleic acid was obtained unexpectedly in attempts to accelerate the action of the hydrolytic enzyme system present in *V. anthelmintica* seed. The purpose was to obtain a high yield of vernolic (epoxyoleic) acid since it had been found[6,7] that trivernolin was the major component obtainable from the seed oil when this enzyme system was inactivated. The (+)-isomer was isolated as follows: freshly ground *V. anthelmintica* seed was incubated at 28° for two weeks under nitrogen and in a water-saturated atmosphere. The meal was thoroughly extracted with petroleum ether (b.p. 39–59°). It was necessary to extract the petroleum ether-extracted meal with diethyl ether in order to obtain all of the lipid constituents. The pure compound was obtained from both extracts by repeated crystallisations from petroleum ether-diethyl ether at 0°, ethyl acetate at 0°, acetone at −20° (first decolourised with Darco G-60) and twice from acetone at 22°. This (+)-isomer, m.p. 63–63·3°, $[\alpha]_D^{27}$ +19·0° (*c.*, 10·0 in EtOH), was isolated in 9·3% yield based on weight of the oil (Found: C, 69·1; H, 10·8. Calc. for $C_{18}H_{34}O_4$: C, 68·8; H, 10·9%), oxirane content, nil.

(−)-*Threo*-12,13-dihydroxyoleic acid, m.p. 62·5–63·0°, $[\alpha]_D^{27}$ −18·6 (*c.*, 10·0) (Found: C, 68·1; H, 10·7%), oxirane content, nil, was prepared from *V. anthelmintica* seed oil by acetolysis[2] and purified according to the method of Chisholm and Hopkins.[3] A 1:1 mixture of the (+)- and (−)-isomers crystallised from diethyl ether-petroleum ether melted at 52·5–53°.

Thin layer chromatography of the methyl esters of the (+)- and (−)-isomers of the *threo*-12,13-dihydroxyoleic acids produced single spots having the same migratory characteristics. Their infrared spectra were identical and showed the typical pattern for an unsaturated dihydroxy-ester having the *cis* configuration at the double bond.

Hydrogenation of the (+)- and (−)-*threo*-dihydroxyoleic acids with 10% palladium in carbon powder as catalyst in ethanol gave the corresponding (+)- and (−)-*threo*-12,13-dihydroxystearic acids, m.p. 97·8–98° and 98·5°, $[\alpha]_D^{27}$ +23·8° and −23·4° (*c.*, 3·0) (Found: C, 68·4; H, 11·4 and C, 68·4; H, 11·5. Calc. for $C_{18}H_{36}O_4$: C, 68·3; H, 11·5%). A 1:1 mixture of the (+)- and the (−)-dihydroxystearic acids, crystallised from ethyl acetate, melted at 98·5–99°.

The results obtained for the (+)- and (−)-*threo*-12,13-dihydroxyoleic and for the corresponding stearic acids are in agreement with those reported by Chisholm and Hopkins.[3]

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